PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project

Genetic Analysis Of Oncorhynchus Nerka (Modified To Include Chinook Salmon

BPA project number: 9009300
Contract renewal date (mm/yyyy): 1/1999 ☒ **Multiple actions?**

Business name of agency, institution or organization requesting funding
University of Idaho

Business acronym (if appropriate) U of I

Proposal contact person or principal investigator:

Name	Madison S. Powell
Mailing Address	3059F National Fish Hatchery Road
City, ST Zip	Hagerman, ID 83332
Phone	(208) 837-9096
Fax	(208) 837-6047
Email address	fishdna@micron.net

NPPC Program Measure Number(s) which this project addresses
4.2A, 4.3C.1, 7.4D.2, 7.5A.1, 8.4A.1, 8.4B.1

FWS/NMFS Biological Opinion Number(s) which this project addresses
NMFS-Hydrosystems Operations Biological Opinion, 1996 NMFS Biological Opinion for IDFG permit # 1010 (Section 10) Captive Rearing of ESA-listed Snake River Salmon

Other planning document references
Endangered Species Act requirements, Snake River Salmon Recovery Plan, Wy-Kan-Ush-Mi-Wa-Kish-Wit (specifically Nez Perce Tribal interests with chinook salmon), 1990 Salmon River Subbasin Salmon and Steelhead Production Plan (objective #6).

Short description
Provide biological and genetic information on O. nerka and O. tshawytscha samples collected throughout the Snake and Columbia Basins to be used in the overall recovery of endangered Snake River sockeye salmon and threatened Salmon River chinook salmon.

Target species
Sockeye salmon (Oncorhynchus nerka) and chinook salmon (Oncorhynchus tshawytscha)

Section 2. Sorting and evaluation

Subbasin
Systemwide

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
☒ Anadromous fish ☐ Resident fish ☐ Wildlife	☒ Multi-year (milestone-based evaluation) ☐ Watershed project evaluation	☐ Watershed councils/model watersheds ☐ Information dissemination ☐ Operation & maintenance ☐ New construction ☒ Research & monitoring ☐ Implementation & management ☐ Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
9107100	Snake River Sockeye Salmon Habitat (Sho-Ban Tribes)	This project provides genetic information for habitat and resource management. The 9107100 project provides tissue samples for this project.
9107200	Redfish Lake Sockeye Salmon Captive Broodstock (IDFG)	This project provides genetic information on an endangered population. The 9107200 project provides tissue samples for this project.
9204000	Redfish Lake Sockeye Salmon Captive Broodstock Rearing and	This project provides genetic information on an endangered

	Research (NMFS)	population. The 9204000 project provides tissue samples for this project.
9606700	Manchester Spring Chinook Broodstock Project (NMFS)	This project will provide genetic information for three threatened populations of chinook salmon. The 9606700 project will provide tissue samples for this project.
9700100	Captive Rearing Initiative for Salmon River Chinook Salmon (IDFG)	This project will provide genetic information for three threatened populations of chinook salmon. The 9700100 project will provide tissue samples for this project.
9801002	Captive Rearing Initiative for Salmon River Chinook Salmon-M (IDFG)	This project will provide genetic information for three threatened populations of chinook salmon. The 9801002 project will provide tissue samples for this project.

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
1997	Identification of a listed sockeye in creel samples and straying sockeye at Manchester	yes
1998	Completion of mitochondrial DNA data set for sockeye	Currently this data is being prepared for publication.
1999	Completion of preliminary nuclear DNA data set for sockeye.	Currently projected to end FY1999 and is on schedule.
	See project history Section 8d for further detail between 1990-1995.	

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Continue outmigrant genetic analysis	a	See objectives, Section 8e below.
2	Continue creel survey genetic analysis	a	See objectives, Section 8e below.
3	Continue analysis on returning sockeye	a	See objectives, Section 8e below.
4	Continue evaluation of additional	a	See objectives, Section 8e below.

	nuclear markers.		
5	Continue evaluation of stray O. nerka	a	See objectives, Section 8e below.
6	Continue evaluation of early and late spawning o. nerka	a	See objectives, Section 8e below.
7	Begin genetic analyses of captively reared and returning chinook salmon from Salmon River	a	See objectives, Section 8e below.

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	1/2000	1/2004	see Section 8e below		15.00%
2	1/2000	1/2004	see Section 8e below		15.00%
3	1/2000	1/2004	see Section 8e below	highly dependent upon anadromous returns	2.00%
4	1/2000	1/2004	see Section 8e below		30.00%
5	1/2000	1/2004	see Section 8e below		8.00%
6	1/2000	1/2004	see Section 8e below		5.00%
7	1/2000	1/2004	see Section 8e below	highly dependent upon returns	25.00%
				Total	100.00%

Schedule constraints
None except the amount of work is dependent on collections of returning sockeye and chinook.

Completion date
2004

Section 5. Budget

FY99 project budget (BPA obligated): $139,434

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	M. Powell, Principle Investigator Senior Scientific Aide Graduate Research Assistant	%49	70,450

Fringe benefits	M. Powell @ 28.5% Senior Scientifc Aide @ 34.5% Graduate Research Assistant @ 1%	%11	16,360
Supplies, materials, non-expendable property	chemicals, pipet tips, tubes, gloves, nucleotide primers, etc.	%6	8,400
Operations & maintenance	equipment service and calibration, UPS shipping, Federal Express, long distance calls/faxing.	%1	2,100
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	none	%0	0
NEPA costs	none	%0	0
Construction-related support	none	%0	0
PIT tags	# of tags: 0	%0	0
Travel	1 professional meeting (AFS) and 12 monthly T.O.C. meetings	%2	2,800
Indirect costs	on campus indirect cost rate @44.7%	%31	44,749
Subcontractor	none	%0	0
Other	none	%0	0
TOTAL BPA FY2000 BUDGET REQUEST			$144,859

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount ($)
		%0	
		%0	
		%0	
		%0	
Total project cost (including BPA portion)			$144,859

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	$145,000	$147,000	$75,000	$75,000

Section 6. References

Watershed?	Reference
☐	Allendord, F.W., and R.S. Waples. 1996. Conservation and Genetics of

	Salmonid Fishes. pp 238-280. In: J.C. Avise, and J.L. Hamrick (eds.). Conservation Genetics: case histories from nature. Chapmena nd Hall, New York.
☐	Banks, M.A., B.A. Baldwin, and D. Hedgecock. 1996. Research on chinook salmon (Oncorhynchus tshawytscha) stock structure using microsatellite DNA. Bull. Natl. Res. Inst. Aquatcult. Suppl. 2:5-9.
☐	Brannon, E.L., A. Setter, T. Welsh, R. Danner, K. Collins, M. Casten, G. Thorgaard, K. Adams, and S. Cummings. 1994. Genetic analysis of Oncorhynchus nerka: life history and genetic analysis of Redfish Lake Oncorhynchus nerka. Completion Report. BPA.
☐	Burgner, R.L. 1991. Life History of Sockeye Salmon (Oncorhynchus nerka). In: Pacific Salmon Life Histories (eds. C. Groot and L. Margolis) University of British Columbia Press, Vancouver. Pp. 3-101.
☐	Dowling, T.E., C. Moritz and J.D. Palmer. 1990. Nucleic acids II: restriction site analysis. In D.M. Hillis and C. Moritz (eds.). Molecular Systematics, Sinauer Associates, Inc., Sunderland.
☐	Everman, B.W. 1895. A preliminary report upon salmon investigations in Idaho in 1894. Bulletin U.S. Fish Commission. 15:253-284.
☐	Federal Register. 1992. 57 FR 213.
☐	Felsenstein, J. 1993. PHYLIP: phylogenetic inference package. University of Washington, Seattle.
☐	Fitch, W.M. and M. Margoliash. 1967. Construction of phylogenetic trees. Science 155:279-284.
☐	McElroy, D., P. Moran, E. Bermingham and I. Kornfield. 1991. REAP: the restriction enzyme analysis package. Center for Marine Studies, University of Maine, Orono.
☐	Moran, P., D.A. Dightman, R.S. Waples, and L.K. Park. 1997. PCR-RFLP analysis reveals substantial population-level variation in the introns of Pacific Salmon (Oncorhynchus spp.). Molecular Marine Biology and Biotechnology. 6(4):315-327.
☒	Morris, D.B., K.R. Richard, and J.M. Wright. 1996. Microsatellites from rainbow trout (Oncorhynchus mykiss) and their use for genetic study of salmonids. Can. J. Fish. Aquat. Sci. 53:120-126.
☐	Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
☐	Ricker, W.E. 1938. “Residual” and kokanee salmon in Cultus Lake. J. Biol. Bd. Can. 4:192-218.

☐	Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular Cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor.
☐	Waples, R.S., O.W. Johnson, and R.P. Jones, Jr. 1991. Status Review for Snake River Sockeye Salmon. U.S. Dep. Commer., NOAA Tech Memo. NMFS F/NWC-195, 23 p.
☐	Waples, R.S., P.B. Abersold, and G.A. Winans. 1997. Population genetic struction and life history variability in Oncorhynchus nerka from the Snake River Basin. Final Report. Bonneville Power Administration.

PART II - NARRATIVE

Section 7. Abstract

This ongoing project seeks to: (a) comprehensively identify the genetic structure of Redfish Lake *O. nerka* outmigrant-originating populations; (b) provide long term information about the genetic identity of returning anadromous sockeye as this run is restored; (c) define the relatedness of populations of *O. nerka* in the Columbia Basin; (d) provide information to monitor the change or loss of genetic biodiversity among *O. nerka* populations throughout the Columbia Basin and in particular, Endangered populations in Redfish Lake, Idaho; (e) begin assessing genetic variation among three Threatened populations of chinook salmon (*Oncorhynchus tshawytscha*) in the Salmon River Subbasin using DNA techniques; (f) provide comparative, long term genetic information as those chinook runs are recovered; and (g) provide genetic information necessary for ongoing captive propagation and captive rearing evaluation programs of both *O. nerka and O. tshawytscha*. The DNA methods used to examine both sockeye and chinook populations have been widely used and can be compared directly with other ongoing or collateral genetic work. This project directly addresses the Columbia Basin Fish and Wildlife Program measures associated with the conservation genetics of threatened and endangered populations and captive broodstock evaluation. This project will be reduced by approximately 50% to a genetic monitoring program for listed sockeye and chinook populations once the database for Salmon River chinook is completed in 2001. The inclusion of Salmon River chinook genetic analysis within this project's scope was determined to be of critical importance by the Chinook Salmon Captive Propagation Technical Oversight Committee.

Section 8. Project description

a. Technical and/or scientific background

Sockeye salmon (*Oncorhynchus nerka*) utilize lake environments for juvenile rearing and differ in that respect from the other species of Pacific salmon (Burgner, 1991). Unique too is the considerable variation observed in sockeye life history patterns or strategies. Although primarily anadromous, there are two life history variants of *O. nerka* that do not migrate to the ocean but instead remain in nursery lake systems throughout maturity. The predominant non-anadromous form, called kokanee, are considered to be wholly freshwater-adapted descendents from a common anadromous stock that diverged during recent geological times. Kokanee populations are numerous throughout the Pacific Northwest and exist in lake environments independently of anadromous sockeye runs. The second non-anadromous form of *O. nerka*, referred to as "residual" sockeye, are regarded as progeny of anadromous adults (Ricker 1938). Residual sockeye populations have a sex ratio heavily skewed toward males, do not develop strong secondary sexual characteristics, and occur sympatrically with anadromous sockeye populations.

Redfish Lake in the Sawtooth Basin of Central Idaho is uncommon among sockeye nursery lakes since it supports populations of all three forms of *O. nerka* (Allendorf and Waples 1996). Anadromous sockeye returning to Redfish Lake travel approximately 1500 km from the Pacific Ocean and spawn at an elevation of 2000 m (Waples et al. 1991). Their extraordinary freshwater migration and the high elevation where Redfish Lake sockeye spawn are distinct from other sockeye runs. Anadromous sockeye return to Redfish Lake in October and November and spawn in shallow gravel beach areas principally along the northeast shoreline of the lake. The native kokanee population is both temporally and spatially separated from the anadromous sockeye population during spawning. The kokanee spawn much earlier, in August and September, and in the major tributary to Redfish Lake, Fishhook Creek (Brannon et al., 1991). The residual sockeye population spawns in the same location and at the same time as do the anadromous sockeye. However, the residual sockeye in Redfish Lake are distinguishable from anadromous sockeye by general appearance. Residual sockeye though similar in size to kokanee, are much smaller than anadromous sockeye (15-20 cm vs. 50-60 cm).

Historical accounts of Redfish Lake *O. nerka* differ from contemporary observations. Evermann (1895) reported large "redfish" spawning in Fishhook Creek during August in 1887-1889 and 1893, indicating the historical sockeye population returned much earlier than the current anadromous stock and that they used Fishhook Creek to spawn in instead of the current beach spawning areas in Redfish Lake. Temporal differences in spawning between the historical sockeye population and the contemporary sockeye population are most likely due to temperature differences between the cooler inlet stream and the warmer shallow beach areas in the lake. Likewise, the present kokanee population that use Fishhook Creek also spawn earlier than current beach spawning populations of anadromous and residual sockeye.

Sockeye returning to Redfish Lake are the only remaining anadromous run of *O. nerka* in the Snake River Basin and have thus been referred to as Snake River sockeye. Present day returns to Redfish Lake are all but non-existent. Since 1991, only 15 anadromous sockeye have returned to Redfish Lake. As a result, this unique population of sockeye was designated an evolutionarily significant unit (ESU) and was listed as a federally endangered species in November 1991(57 FR 213: 1992). The small, residual

sockeye population in Redfish Lake was also given protection under the Endangered Species Act (ESA) because they are considered to be, at least in part, progeny of listed, returning adults. The sympatric kokanee population in Redfish Lake was excluded from the ESA listing.

From its inception, a primary concern of the Snake River sockeye recovery program has been the clarification of genetic relationships between sympatric populations of *O. nerka* in Redfish Lake. Waples et al. (1997) completed a genetic study of the lake using allozymes. This remaining population of sockeye salmon in the Snake river is considered unique in that their genetics and life history patterns are divergent, to a sufficient degree, from other stocks of sockeye salmon. However, the recovery of this sockeye population to sustainable levels requires genetic monitoring because long term survival will be influenced not only by the restoration and preservation of their habitat but, also by their ability to genetically contend with their historically stochastic environment. This project is essential to all recovery strategies concerned with the maintenance of genetic variation and diversity within this species. Thus, this project directly addresses concerns outlined by the CBFWP.

Chinook Salmon life history and scientific background are reviewed in the FY 1998 BPA project intitled Columbia River Chinook salmon and Steelhead Population Structure (Drs. Powell and Brannon are co-PIs) and for brevity will not be reviewed again here. As outlined above and in subsequent sections the purpose for including chinook salmon under the scope of this ongoing project is to provide genetic information concerning captive rearing technology and its impacts on the three listed populations it is directed at. The methods used are exactly the same as the methods used to evaluate chinook populations in the FY 1998 project above and also used in describing genetic variation among cryopreserved chinook salmon sperm (this includes several contract projects to the PI's).

b. Rationale and significance to Regional Programs

Since the Snake River sockeye and chinook as listed under the endangered Species Act are anadromous, their protection and recovery fall under the jurisdiction of the National Marine Fisheries Service. The broodstock and captive rearing programs are conducted by the National Marine Fisheries Service (program # 9204000 and #9606700) and the Idaho Department of Fish and Game (program # 9107200, #9700100). Our objectives are commensurate with the responsibilities and objectives of the fore mentioned agencies as well as those of the Sho-Ban Tribe (program # 9107100) and the IDFG evaluation of chinook captive rearing demonstration technology (program #9801002). Thus far this has led to a successful, cooperative, interdisciplinary effort toward the conservation of these threatened and endangered species. Our general objectives are directed at resolving the origins and phylogeographic relationships of existing *O. nerka* and *O. tshawytscha* stocks in the Columbia Basin. Additionally, we wish to determine what level of gene flow exists between populations and what genetic contributions are made by anadromous *O. nerka* and resident *O. nerka* populations to reciprocal migratory forms. The results of this research will influence management

decisions regarding the uniqueness of various sockeye and chinook stocks and their designation as evolutionary significant units (ESU). This project will also serve to evaluate captive broodstock and captive rearing technologies as a means of conserving genetic diversity. This work has far reaching implications regarding the Endangered Species Act as well as future conservation efforts for non-endangered populations of trout and salmon.

c. Relationships to other projects

The project provides genetic information to fisheries and resource managers to aid in the restoration and recovery of sockeye and kokanee populations in the Columbia River Basin. Moreover, it will also provide the same type of information on chinook salmon in the Salmon River Basin. This project will result in a comprehensive data base and genetic profile from which the immediate and long term genetic risks to Snake River sockeye and Salmon River chinook salmon can be addressed. The present target populations are listed as federally threatened or endangered species. Several BPA projects and CBFWP measures (listed above in Sections 1 and 3) are directly associated with this project.

d. Project history (for ongoing projects)

Sockeye Project History

Activities have included rearing Redfish Lake kokanee to examine inter-year temperature unit variability for egg incubation and behaviorally for downstream migration volitionally out of circular tanks. Primarily work has focused on defining a technique and regions of DNA base sequences useful for separating the life history forms of *O. nerka* in Redfish Lake. Costs have included renovation of experimental wet laboratory facilities, equipment for genetic laboratory, salaries and supplies. Benefits are that regions useful for diagnostic purposes have been identified for the anadromous versus non-anadromous components of Redfish Lake *O. nerka* and a wet lab that can be used for small scale incubation and rearing projects. Isolation of a single locus nuclear probe which could distinguish the "a" allele seen primarily in Redfish Lake kokanee. The mtDNA information appears to resemble the information collected from the nuclear single locus probe suggesting that the mtDNA information accurately reflects gene flow among the subgroups. Life history characteristics of the three forms were assessed with some differences in development rate of eggs and number of gill rakers counts. DNA analysis included assessment of other *O. nerka* stocks in the Salmon/Snake River system, the Upper Columbia River and outside the Columbia River system. Development of DNA nuclear markers or probes is still underway that might readily segregate the three forms. Preliminary results indicate three forms closely related, but may be sufficiently different to be considered three separate stocks.

Reports/Publications: Genetic Analysis of Oncorhynchus Nerka - Annual Progress Reports FY 1991, FY 1992, Genetic Analysis of O. Nerka: Life History and Genetic Analysis of Redfish Lake O. Nerka, Completion Report FY 1993-1994. Monthy Progress Reports 1991 to 12/1998 and Stanley Basin Sockeye Technical Oversight Committee Meeting Notes 1993-12/1998. Genetic Guidelines for Captive Broodstock and Captive Rearing Strategies- In preparation (due 1999).

Chinook Project History

To date the University of Idaho has gathered and archived 1218 samples from 26 chinook populations. Genetic analyses were used (explained in methods Section 8f) in 1998 to construct a genetic dissimilarity matrix for IDFG to identify crosses to be made between captively spawned East Fork Salmon River chinook. The utility of this data was presented and demonstrated at the 1998 Northwest Fish Culture Conference and in being prepared for peer-reviewed publication.

e. Proposal objectives

The following objectives are directed at resolving the genetic relationships of existing stocks of *O. nerka* in the Columbia Basin and listed *O. tshawytscha* populations in the Salmon River Subbasin with the goals of determining what level of divergence exists between populations. The ongoing analysis and proposed future work will be used to test the following hypotheses:

1) Anadromous, Redfish Lake sockeye salmon are a distinct population of *O. nerka* and represent an evolutionarily significant unit (ESU) recognized under the Endangered Species Act and are following an independent evolutionary trajectory as evidenced by reduced gene flow relative to a sympatric kokanee population.

2) Beach spawning, resident sockeye are an intermediate form between anadromous sockeye and kokanee but, most closely resemble anadromous sockeye in a taxonomic sense leading to their inclusion in the Redfish Lake ESU.

3) The sympatric kokanee population present in Redfish Lake is not significantly contributing to the numbers of outmigrating anadromous sockeye and thus the Redfish Lake ESU. Populations of *O. nerka* within the Columbia and Snake River basins form genetically distinct stocks due to their high degree of philopatry and thus may give rise to additional situations where a sockeye populations become reduced in number and have to be considered for protection under the ESA.

3) The genetic diversity of listed Salmon River chinook salmon populations are not significantly affected by captive rearing strategies and overall genetic diversity and identity is not significantly different from the remaining wild returns.

Alternative Rationale:

Alternative approaches to accomplishing this projects objectives arise in the form of alternative approaches to the gathering and analysis of genetic data. The particular methods used in this project have been selected based upon their informativeness, reproducibility, cost effectiveness, and productivity.

Study Plan:

This ongoing investigation is separated into three areas of interest; **(a)** identification of genetic similarities and differences among populations of *O. nerka* in Redfish Lake with emphasis on ESA listed populations; **(b)** genetic analysis of anadromous and non-anadromous populations of *O. nerka* in the Columbia River Basin; and **(c)** genetic analysis of ESA listed Salmon River chinook salmon populations.

Objectives:

The Objectives of the 2000 study are to:

1. Continue DNA analyses on tissue samples from outmigrant assemblages (from Redfish, Alturas, and Pettit Lakes) to assess contributions from kokanee and resident sockeye populations on subsequent year classes. These genetic studies will include the use of mitochondrial DNA RFLP analyses we have previously employed.
2. Continue screening tissue samples from Redfish Lake creel surveys to assess potential harvest of listed, resident sockeye and to establish baseline information on the genetic diversity of kokanee harvested.
3. Continue DNA analyses on tissue samples of captive broodstock progeny for comparison to returning sockeye assemblages and thus, characterize successful outmigrant contributions.
4. Continue to evaluate nuclear DNA markers for their utility in distinguishing populations of *O. nerka*.
5. Continue to examine various tissue samples of interest to evaluate the origin of stray *O. nerka* in the Columbia River Basin.
6. Continue the analysis of genetic differences and population substructure among early and late spawning *O. nerka*.
7. Begin genetic analysis of captively reared Salmon River chinook salmon.

Objectives 1 and 3: Monitoring Redfish Outmigrants and Returns

One of the objectives of any recovery plan should address the long term stability of the listed population(s), in this case Redfish Lake anadromous and resident sockeye salmon. The primary task of this ongoing project is to monitor the genetic diversity of anadromous adults returning to Redfish Lake to spawn and the concomitant diversity of outmigrating sockeye smolts. The reduced numbers of these fish have increased the probability that minor stochastic events and random genetic drift may reduce or eliminate genetic diversity within the listed populations. Captive propagation of Redfish Lake

sockeye is well underway and we have used mtDNA RFLP analysis to identify maternal lineages of sockeye in the past. This genetic information has aided broodstock managers in making genetic crosses and will play an increasingly important role in future decisions regarding crosses and "safety net" broodstock programs. It is also important to identify likely sources of genetic variation from other closely related populations in the event out breeding becomes necessary in the captive breeding program.

Objective 2: Monitoring Redfish Lake Creel Samples

In 1996-97, tissue samples from 49 individual *O. nerka* obtained during creel surveys were analyzed using mtDNA RFLP analysis. Seven of 8 composite haplotypes observed among the creel samples were shared with composite haplotypes found among Fishhook Creek kokanee. The remaining haplotype, designated H07, has not been observed in Fishhook Creek kokanee (N=81) but, has been identified in beach spawning sockeye (N=22). This indicates the incidental take of a listed resident sockeye and points out the need for continued genetic monitoring of creel surveys from Redfish Lake. Furthermore, 2 of the 8 composite haplotypes observed in the creel samples are also shared among both Fishhook Creek kokanee as well as resident sockeye. We are unable to distinguish resident sockeye from kokanee that share these two haplotypes. Thus, there is a possibility that additional resident sockeye were taken during the kokanee season. Greater resolution in our genetic analyses is needed to confirm this possibility and will only be accomplished through the development of discriminatory nuclear DNA markers.

Objective 4: Nuclear DNA Marker Development

The development of a suite of polymorphic nuclear markers is essential to the continued success of any genetic monitoring program for Redfish Lake listed populations. Genetic diversity among captive *O. nerka* broodstock must be further characterized with greater resolution. Thus far, this project has successfully used mtDNA RFLP analysis to identify different maternal lineages of *O. nerka* in Redfish Lake populations and recommend possible management strategies but, in most every case, greater discriminatory power would enhance management decisions.

Objective 5: Identifying Stray *O. nerka*

During 1996 and 1997 anadromous sockeye were sampled at locations unusual for returns. These locations included the outlet for the NMFS facility at Big Beef Creek, the Lochsa River, and the Pelton Dam fish trap on the Deschutes River. Genetic analysis on these fish revealed the sockeye returning to Big Beef Creek were not of Redfish Lake captive broodstock origin and unlikely to be of Wenatchee Lake broodstock origin, as was originally presumed. The female sockeye sampled from the Lochsa River had a composite haplotype common to most sockeye populations but absent from Redfish Lake anadromous and resident sockeye populations. The sockeye returning to the Pelton Dam fish trap were observed to contain a composite haplotype that has not appeared in samples from other populations. Straying among salmonids is a natural phenomenon. Unfortunately, the origin(s) of the "stray" sockeye remain unknown. The fish returning to Big Beef Creek and the Lochsa River have composite haplotypes common to many anadromous populations. The resolution of our current mitochondrial markers is

insufficient to pinpoint where the fish originated. We will continue to provide timely, genetic information on "stray" sockeye to other agencies involved in the Snake River sockeye recovery effort. We also intend to use nuclear markers we are currently developing to enhance the resolution of our analyses.

Objective 6: Analysis of Early and Late Spawning Population Substructure

We are continuing our analysis of genetic differences between early and late spawning sympatric populations of kokanee. Gene flow may be reduced between these temporally isolated subpopulations. Differences in composite haplotype frequency or differences in observed composite haplotypes may indirectly reveal reduced gene flow in these populations. In Redfish Lake, an early spawning population of kokanee in Fishhook Creek (N=42) has been characterized with 2 composite haplotypes that have not been observed in other populations of *O. nerka* thus far examined (N>1400). These 2 uncommon haplotypes have also been observed in the Redfish Lake creel surveys (N=49). Late spawning Fishhook Creek kokanee also contain 2 composite haplotypes that have only been observed in that subpopulation but, these 2 uncommon haplotypes have not been observed in the Redfish Lake creel surveys. Creel samples from Redfish Lake also appear to be most similar to early spawning Fishhook Creek kokanee when composite haplotype frequencies are compared.

Objective 7: Begin analysis of Salmon River Chinook Salmon.

Adults returning to the Lemhi River, the West Fork Yankee Fork Salmon River, and the East Fork Salmon River will be non-destructively sampled along with captive adults and juveniles currently at the Manchester Laboratory and the Eagle Fish Hatchery facilities. The returns, spawning adults and progeny will be examined for significant differences and similarities within there mitochondrial and nuclear genetic diversity. They will also be compared to existing data on other chinook populations to place the observed genetic differences or similarities in context with other populations.

f. Methods

Two DNA sources have been used by this laboratory to study genetic differentiation within and among *O. nerka* and *O. tshawytscha* populations, mitochondrial DNA and nuclear DNA. The methods used encompass both repetitive and non-repetitive sequences, as well as coding and non-coding sequences. Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) has been primarily used because of it's cost effectiveness and relatively straightforward application. The reason for using nuclear DNA markers in this project has been to compliment the existing mitochondrial DNA database for sockeye and the growing database for chinook as well as add to the confidence and precision of our present conclusions. Nuclear DNA markers potentially offer a much higher level of resolution regarding genetic variation and differentiation. Using genetic data from separate sources, such as mitochondrial and nuclear DNA, strengthens the results of each and reduces potential bias in conclusions drawn from using only one data set. It should be noted that

an assumption has been made regarding the long term survival of Snake River sockeye and chinook salmon and their genetic adaptability. We have proceded with the assumption, until proven otherwise, that sufficient genetic variation exists within the anadromous, beach spawning, and/or sympatric kokanee population (in *O. nerka*) to address that organism's natural environmental challenges to survival. Moreover, we have proceded on the assumption that similar variation exists in returning Salmon River chinook The level of genetic variation needed changes with the organism under study and with the stability of its environment. Some organisms thrive under conditions of little genetic diversity, but others do not. Consequently, their risk of extinction can be very high. At this point, the level of genetic variation needed in the Snake River sockeye population for good probability of long term survival is unknown. This is also true for the threatened populations chinook salmon in Idaho. Likewise, questions remain as to whether or not a sufficient level of genetic variation still exist. The genetic analyses undertaken in this project will help to facilitate an answer to both those questions. A critical assumption to each hypothesis is that the samples analyzed for each population are representative and unbiased. The chance that this problem will occur can be minimized by increasing the number of individuals tested from each population. At present, we are continuing to expand the data base to include where possible, 60 individuals from each population under study. Statistically, the distribution of mitochondrial haplotypes found among each population will then fall within 95% confidence limits for haplotypes that are not considered "rare." (i.e. with a sample size of 60, there is a 95% chance of observing every haplotype in a population that occurs with 5% or greater frequency). There are no known risks associated with non-destructive sampling and genetic analysis of *O. nerka* or *O. tshawytscha* populations. Genetic data from this project will be analyzed using several commomly used statistical programs for this purpose. They include, NTSYS, PHYLIP, PAUP, Sigma STAT, DNAsize, Sigma Scan and several others. The outcomes will be evaluated by project staff as well as members of the Technical Oversight Committee and NMFS.

Sockeye and Chinook Mitochondrial DNA Analyses

We employ the polymerase chain reaction (PCR) to amplify four separate gene regions of mitochondrial DNA, Cytochrome b, NADH dehydrogenase subunit 1, NADH dehydrogenase subunit 2, and NADH dehydrogenase subunits 5/6. These sequences of DNA code for proteins active in the oxidative-phosphorylation pathway, carried out within the mitochondrial matrix. The DNA found in mitochondria is circular in conformation, small (approximately 16,000 base pairs in size), maternally inherited, and non-recombinatory. Mitochondrial DNA exhibits a relatively rapid rate of nucleotide change or evolution (when compared to nuclear gene sequences). For these reasons, mtDNA has been widely applied in studies of population genetics and phylogeography. Tissue samples from each fish are stored separately in 70% ethanol or lysis buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 50 mM EDTA, 1% Sodium dodecyl sulfate, 0.2% Dithiothreitol) until DNA was extracted using methods modified from Sambrook et al. (1989) and Dowling et al. (1990). The polymerase chain reaction (PCR) was used to amplify sequences (Figure 2) from each DNA sample using nucleotide primers specific for the mitochondrial Cytochrome b and NADH dehydrogenase subunit 1, 2, and 5/6 gene regions (LGL Ecological Genetics). Amplified mtDNA gene regions were digested

using 13 Type II restriction endonucleases (Table 2). The resulting mtDNA fragments were separated by electrophoresis using agarose or polyacrylamide gels. Gels were stained with ethidium bromide and restriction fragment patterns visualized using UV light. Photographs of each gel were converted into computer image files using a ScanMan scanner and ScanMan 2.0 software (Logitech). Restriction fragment length polymorphisms (RFLPs) observed among samples were measured using SigmaScan Pro 2.0 (Jandel Scientific 1995), then given alphabetical designations as simple haplotypes. Fragment sizes of each RFLP from each gene region were estimated by comparison to a size standard, pUC-19 marker (Bio-Synthesis). Alphabetical designations from RFLPs of each mitochondrial gene region were combined into composite haplotypes. An estimate of the number of nucleotide substitutions per site (p) for each RFLP was calculated via the Nei (1987) method using REAP 4.0 (Restriction Enzyme Analysis Package) (McElroy et al., 1991) then used to generate a matrix comparing p values (distance) between all pairs of identified composite haplotypes. The KITSCH program in PHYLIP 3.5 (Felsenstein 1993) which assumes independence and equal rates of divergence was used to generate an distance dendrogram via the least-squares method of Fitch and Margoliash (1967) to illustrate the estimated evolutionary relationships and distance among the identified composite haplotypes.

Sockeye Nuclear DNA Analyses

This project has examined several types of nuclear DNA markers for their utility in delineating *O. nerka* populations. Our current research in this area includes the use of 20 DNA primers constructed from a cloned, partial genomic library of *O. nerka* (courtesy of Dr. Kim Scribner). These nuclear primers are used to PCR amplify microsatellite sequences variable in size and sequence among and within populations. We are also using PCR to amplify two separate nuclear gene regions, p53 and growth hormone II (similar to chinook nuclear gene regions). Primers for these two regions were obtained from Drs. Linda Park and Paul Moran of NMFS and were originally designed for use with chinook salmon. These two nuclear gene sequences exhibit restriction fragment length polymorphisms when different *O. nerka* populations are compared. We are currently modifying three additional sets of primers from nuclear gene sequences for use with this study. A third area of investigation involves amplified fragment length polymorphisms (AFLPs) and uses nuclear DNA primers to amplify 100-200 loci in a single PCR reaction. This method is potentially more cost effective than some other methods and provides a greater probability of detecting polymorphisms with limited sample sizes.

Chinook Nuclear DNA Analyses

This project will use the same two primer sets listed above for the p53 gene and growth hormone II gene sequences. The sets were originally developed from chinook salmon and work extremely well. Additionally, modified primer sets for two microsatellite sequences, PuPuPy and Omy77 (from Jonathan Wright's laboratory) will be used to PCR amplify these repetitive sequences. Both of these types of nuclear DNA sequences, coding (p53 and growth hormone II) and non-coding (microsatellites) have already been used analyze approximately 20% of our existing chinook sample inventory and their utility has been demonstrated (Banks et al., 1996; Park et al., 1996; Morris et

al., 1996; Moran et al., 1997). Using information from these sequences as well as mtDNA genetic information provide a statistically robust data set for analysis and comparison. The statistical programs used to evaluate nuclear data include BIOSYS-1 (Swofford and Selander, 1981) and other similar programs such as Genepop (shareware).

g. Facilities and equipment

The Aquaculture Research Institute (ARI) at the University of Idaho directed by Dr. E. Brannon, maintains a fisheries genetics laboratory. This facility has two full time lab technicians, a full time research scientist (Dr. M. Powell), a half time doctoral research assistant, and contains all the equipment necessary to collect, generate, and analyze molecular genetic data necessary for the ongoing project. This includes all laboratory equipment, data analysis sofware, office, and clerical support. The University of Idaho's Hagerman Fish Culture Experiment Station (HFCES), with funding from the National Science Foundation (NSF EPSCoR # EPS-9632684), created the Salmonid and Freshwater Fish Research Laboratory. This laboratory is primarily a molecular genetics facility and in conjunction with the ARI fisheries genetics laboratory has completed preliminary examinations of mitochondrial DNA among sockeye. Genetic analyses are divided between the two facilities to expedite the completion of this project. The majority of the nuclear DNA analysis is conducted at the HFCES Salmonid and Freshwater Fish Research Laboratory. The remaining mitochondrial DNA analysis is performed at the ARI genetics facility.

No field equipment costs or tissue collection is necessary during this project. All tissue samples required have been collected by coordinating agencies (ODFW, WDFW, IDFG, and SBT) or are listed for yearly ongoing collection under their current budgets.

The University of Idaho's Aquaculture Research Institute, specifically the fisheries genetics laboratories, provide a centralized archive of fish tissue samples for molecular systematic evaluation. The ARI's goal is to generate useful and necessary population genetic data for the benefit of all managers, agencies, and tribes. Currently the University of Idaho has collected, under the auspices of this project and others, over 3400 tissue samples of *O. nerka* comprising 32 separate populations and 1218 samples of chinook salmon comprising 26 populations throughout the Pacific Northwest and British Columbia.

h. Budget

The budget for this project includes salary for personnel which has increased by 5% over FY1999, a university mandated salary increase for all employees. Fringe benefits remain at the same rate of 28.5% for Dr. Powell and the Sr. Scientific Aide and 1% for the Graduate Assistant. Supplies and Materials, and Operations and Maintenance reflect previous levels of funding. There are no Capital acuisitions, NEPA, Construction-related support, or PIT tag costs associated with this project. Travel costs represent attendance at monthly Technical Oversight Committee meetings and a presentation of data at 1 professional meeting. Indirest costs are listed under the on campus rate of 44.7% since a majority of the work is being performed at the ARI fish genetic laboratory (Moscow).

Section 9. Key personnel

Name	Employer	Title	FTE/hours
Ernest L. Brannon	Univ. of Idaho	Project Coordinator	0
Madison S. Powell	Univ. of Idaho	Research Scientist	0.5/2080

Duties for this project and qualifications for the proposed work:

All of the key personnel involved in this project have previously worked with and are currently contracted for this ongoing project. Dr. Brannon has previously published on genetic variaton in Snake River sockeye salmon (Brannon et al., 1994). Drs. Powell and Brannon are supported with funding from a National Science Foundation grant to examine Snake River sockeye and chinook salmon until June 1999. Drs. Powell and Brannon are also supported by a BPA grant (project # 9800403) to examine genetic diversity among chinook salmon and steelhead in the Columbia River Basin. That BPA project will end in Oct. 1999. All the procedures to be used in this ongoing project are either currently being employed (mitochondrial RFLP variation and sequence variation and microsatellite analysis etc.). Dr. Powell and contracted laboratory personnel conduct the laboratory work. Drs. Brannon and Powell analyze the data generated and interpret the results as they apply to sockeye salmon and chinook salmon management and conservation.

Curriculum vitae for key personnel follow:

MADISON S. POWELL

Education:
Ph.D., 1995, Texas Tech University
M.S., 1990, University of Idaho
B.S., 1985, University of Idaho

Current employer: University of Idaho, Hagerman Fish Culture Experiment Station
3059 F National Fish Hatchery Road, Hagerman, ID 83332, (208) 837-9096
FAX: (208) 837-6047, email fishdna@micron.net

Current Responsibilities: Research scientist; supervise fisheries genetics laboratories and lab personnel at the Aquaculture Research Institute and the Hagerman Fish Culture Experiment Station.

Previous employment:

1997-present	Research Scientist, Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho
1996-1997	Research Scientist, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995-1996	Postdoctoral Fellow, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995	Ph.D., Zoology, Texas Tech University
1990	M.S., Zoology, University of Idaho
1985	B.S., Zoology/Biology, University of Idaho

Technical experience:

DNA and RNA isolation, molecular cloning, genomic libraries, DNA fingerprinting, automated sequencing, PCR amplification, RFLP analysis, RAPD analysis, *in vitro* transcription, fluorescence *in situ* hybridization, karyotyping, cell and tissue culture, nucleotide and protein electrophoresis, liquid chromatography, HPLC analysis, small animal surgery, field collection, and identification.

Five publication closely related to this project:

Williams, R.N., M.S. Powell, R.P. Evans, and D.K. Shiozawa. 1998. Genetic Analysis of Putative Yellowstone Cutthroat Trout samples from the Henry's Fork Subbasin. Center for Salmonid and Freshwater Species at Risk, University of Idaho. Technical Report. Pp 1-9.

Powell, M.S. V.L. Paragamian, and J.C. Faler. 1998. Genetic characteristics of burbot in the Kootenai River drainage of Montana, Idaho, and British Columbia. Proceedings of the International Congress on the Biology of Fish. Burbot Symposium. Pp. 1-4.

Anders, P. and M.S. Powell. 1998. Comprehensive management and conservation of Columbia Basin white sturgeon (*Acipenser transmontanus*): A zoogeographic approach. Proceedings of Ecosystem Based Management in the Upper Columbia River Basin. Pp. 53-54.

Paragamian, V.L., M.S. Powell, J.C. Faler, and S. Snelson. (accepted for publication) Mitochondrial DNA analysis of burbot *Lota lota* stocks in the Kootenai River Basin of British Columbia, Montana, and Idaho. *Trans. Amer. Fish. Soc.*

Powell, M.S. and J.C. Faler. Genetic differentiation among early and late spawning populations of kokanee salmon. In preparation, *Can. J. Fish and Aquat. Sci.*

ERNEST L. BRANNON

Education:
Ph.D., 1973, Fisheries, University of Washington
B.S., 1959, Fisheries, University of Washington

Current Employer/Responsibilities:
Director, Aquaculture Research Institute, University of Idaho
State Aquaculture Extension Specialists
Professor of Fish and Wildlife Resources
Professor of Animal and Veterinary Sciences

Professional experience:

1988-present:	Director, Aquaculture Institute, University of Idaho, Moscow, Idaho
1984-1988:	Professor, School of Fisheries, College of Ocean and Fisheries Sciences, University of Washington, Seattle
1974-1983:	Director, Finfish Aquaculture Program, College of Fisheries, University of Washington, Seattle, Washington
1973-1975:	Assistant Professor, College of Fisheries, University of Washington, Seattle
1971-1972:	Chief Biologist, International Pacific Salmon Fisheries Commission (IPSFC), New Westminster, B.C., Canada
1969-1971:	Supervisor, Sockeye Management Research, IPSFC, New Westminster, B.C., Canada
1959-1969:	Research Biologist, Fisheries Management, Artificial Propagation, Spawning Channel Development and Fish Culture, IPSFC, New Westminster, B.C., Canada
1953-1959:	Field Management, IPSFC, New Westminster, B.C., Canada

Five publications closely related to the proposed project

Powell, M.S. G.H. Thorgaard, R.L. Williams, B.A. Robison, J.C. Faler, and E.L. Brannon. Genetic analysis of sockeye salmon (*Oncorhynchus nerka*) in Redfish Lake. Annual Completion Report, U.S. Dept. of Energy, Bonneville Power Administration, Portland. In preparation.

Cummings, S.A., E.L. Brannon, K.J. Adams, and G.H. Thorgaard. 1997. Genetic Analysis to Establish Captive Breeding Priorities for Endangered Snake River Sockeye Salmon. Conservation Biology 11(3):662-669.

Brannon, E.L. and A.W. Maki. 1996. The *Exxon Valdez* Oil Spill: Analysis of Impacts on the Prince William Sound Pink Salmon. Reviews in Fisheries Science 4(4):289-337.

Thorgaard, G.H., P. Spruell, S.A. Cummings, A.S. Peek, and E.L. Brannon. 1995. Mixed DNA fingerprint analysis differentiates sockeye salmon populations. *Pages 295-303 in* J.L. Nielsen and D.A. Powers, editors. Evolution and the aquatic ecosystem: Defining unique units in population conservation. Proceedings of the American Fisheries Society symposium 17 (May 23-25, 1994, Monterey, CA).

Brannon, E. and A. Setter. 1992. Movements of white sturgeon in Lake Roosevelt (1988-1991). Final Report, Contract # DE-BI79-89BP7298, Project # 89-44, to the US Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, OR. 35 pp.

Section 10. Information/technology transfer

Information generated by this project will be published as peer-reviewed publications and BPA annual reports. Information will also be updated and presented at future Stanley Basin Technical Oversight Committee meetings, Chinook Salmon Captive Propagation Technical Oversight Committee meetings, American Fisheries Society conferences, and BPA project summary conferences. It is critically important for sockeye and chinook salmon management in the Pacific Northwest that information from this project be distributed so that the implications of the results and conclusions can be throughly discussed and reviewed. This project does not involve nor, does it contain funding for public relations activities. However, this project is funded at a major land-grant university within the region and at a research center that is substantially involved in extention and outreach services. These conditions predicate a certain degree of "visibility" for BPA involvement and concern for the region's efforts to protect, mitigate, and enhance fish and wildlife. Public awareness is also increased by participation of project staff in regional and national scientific meetings and publication in both peer reviewed and non-peer reviewed literature.

Congratulations!